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БІОЛОГІЧНА СЕКЦІЯ – «ВАЖЛИВІСТЬ ІДЕЙ Г. А. ГАМОВА ДЛЯ БІОЛОГІЇ 21-ого СТОЛІТТЯ» XXI МІЖНАРОДНОЇ ГАМОВСЬКОЇ КОНФЕРЕНЦІЇ-ШКОЛИ, ЩО ВІДБУВАЛАСЯ У СЕРПНІ 2021 р. В ОДЕСЬКОМУ НАЦІОНАЛЬНОМУ УНІВЕРСИТЕТІ ІМЕНІ І.І. МЕЧНИКОВА

Третій рік поспіль в межах Гамовської конференції працює Біологічна секція: «Важливість ідей Г.А. Гамова для біології 21-ого століття», організація якої викликана великою повагою до особистості вченого Георгія Антоновича Гамова, наукові інтереси якого об'єднали астрофізику, космологію та молекулярну біологію.

Цього року Біологічна секція працювала в режимі on-line 17.08.2021.

Роботу секції розпочали з виступу професора Тобіуса Дельбрюка (Institute of Neuroinformatics – ETH and University of Zurich, Zurich, Switzerland), сина видатного фізика, Нобелевського лауреата Макса Дельбрюка (в певний період товариша Г.А. Гамова). Професор Тобіус Дельбрюк назвав свою доповідь – «Out of this world: Recounting Max's Delbruck memories of George Gamow". Ця доповідь, присвячена феноменальній особистості Г.А. Гамова, придала засіданню біологічної секції емоційну атмосферу наближення до великих ідей, що надали і зараз надають поштовх для розвитку молекулярної біології.

Значну зацікавленість учасників секції викликала доповідь Dr. V.N. Korzun (KWS SAAT SE & Co. KGaA (м. Айнбек, Німеччина) «Applications of genetic and genomic research in cereals», що продемонструвала впровадження в селекційний процес сучасних молекулярно-генетичних технологій. З доповіддю «DNA-protein interactions as a tool of synthetic biology», що присвячена високо технологічним розробкам зі створення біосенсорів науково-виробничою фіромою Explogen LLC (EXG) (м. Львів, Україна) виступив к.б.н. Ю. Ребець. Наступна пленарна доповідь «Using the G.A. Gamow's ideas for molecular genetic diagnostics of infectious and somatic human diseases at the current stage of medical development» була представлена білоруськими вченими, а саме професор С.А. Костюк розповіла про молекулярно-генетичні дослідження, що виконуються в Білоруській медичній академії післядипломної освіти. Dr. Yu. Monchak з McGill University (м. Монреаль, Канада) також представив доповідь присвячену впровадженню ДНК-технологій в діагностику патології людини – «Targeted therapy, DNA sequence and the race against neoplasia». Ця доповідь викликала жваве обговорення.

Молоді науковці Іщенко О.О., Жарікова Д.О., Роман І.І., Доля Б., Рошка Н.М., Чубик І.Ю., Попович Ю.А., Топораш М.К., Пидюра М.О.– доктори філософії з біології, кандидати наук, аспіранти, що займаються дослідженнями в галузі молекулярної біології представили дев'ять доповідей, що відбивають результати виконаних досліджень у низці провідних університетів нашої країни, а саме у Львівському національному університеті імені І. Франка, у Одеському національному університеті імені І.І. Мечникова, у Чернівецькому національному університеті імені Юрія Федьковича та ДУ «Інституті харчової біотехнології та Геноміки» (м. Київ). Представлені доповіді викликали жвавий інтерес, а формат on-line дозволів об'єднати у роботі секції понад 35 учасників з різних країн – України, Білорусі, Швейцарії, Німеччини, Італії, Канади і Казахстану.

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APPLICATION OF GENETIC AND GENOMIC RESEARCH IN CEREALS

The growing population of the 21st century is placing increasing demands on our food production, this is compounded by pressures from diminishing resources and more variable growing conditions. In Europe farmers and the agricultural industry also have to navigate the clearly defined targets from the EU Green Deal Strategy. In this context, genomics and associated molecular marker technology, and genome editing must play an important role in developing new varieties that are better adapted to address these key challenges.

Great advances have been made in recent years in systems to detect DNA variation and in the technologies used to identify DNA markers linked to useful traits. More recently, single nucleotide polymorphism (SNP) markers have been developed for major cereals and this marker system is predicted to accelerate advances in both marker development and implementation in breeding programs. Identification of markers linked to useful traits has been based on complete linkage maps, genetic mapping or/and on the whole genome wide association studies (GWAS). Use of genomic selection (GS) can greatly speed up the selection process and increase precision. In the presented work, the development of molecular genetic maps, application of molecular markers for characterisation of genetic resources, identification of abiotic and biotic stress tolerance loci in cereal crops and selected applications in breeding are presented and discussed. Further prospects for increasing the efficiency of agriculture are associated with the inclusion of high-throughput techniques and new tools for genome editing in the breeding strategy.

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DNA-PROTEIN INTERACTIONS AS A TOOL OF SYNTHETIC BIOLOGY

The progress in molecular biology, genetics and genomics has led to the clear understanding of basic mechanisms of genetic information realization. With this the biological science is transitioning from the era of analysis to the next step in its development – synthesis. This period is characterized by the utilization of available knowledge in order to build new unnatural biological devices with the predictable and controllable features and behavior.

The screening for new natural products with the certain chemical structure or activity is of a key goal of biology of antibiotics producing Actinobacteria. The currently used methods are mostly based on utilization of analytical instruments. Unfortunately, they do have a serious limitation first of all due to low throughput and poor possibilities for multiplexing. The synthetic biology makes it possible to overcome these limitations by development of compound-specific biological sensors.

Herein, we present the design, construction and tuning of the antibiotic-specific cell-based biosensor for the potent antimycobacterial compound pamamycin. The biosensor is built on the transcription factor PamR2 specifically interacting with the target compound. As a signal output part, the reporter gene and pamamycin-regulated promoter are used. The basic design had a low dynamic range and high noise to signal ration. The deep studies of the PamR2 functionality made it possible to replace the natural parts of the signal output module with the synthetic one, dramatically improving the biosensor performance. Solving the structure of the pamamycin-bound PamR2 allowed us to further improve the biosensor performance by shrinking the ligand-binding pocked and thus decreasing PamR2-pamamycin affinity. This led to the increase in operating range of the biosensor. As a result, the construct with the low signal to noise ratio and broadened range of concentration recognized by the sensor was built. The biosensor was utilized in the screening program to identify new producers of pamamycins.

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TARGETED THERAPY, DNA SEQUENCE AND THE RACE AGAINST NEOPLASIA

Recent discoveries, molecular characterizations and DNA sequencing of tumor-specific cell markers paved the way for the synthesis of novel therapeutic agents targeting specifically neoplastic cells without damaging normal tissue. The paradigm of targeted therapy in human anti-neoplastic treatment was imatinib [1], which revolutionized the approach to CML therapy, and ushered in the era of molecular medicine.

The cornerstone of targeted therapy is the presence of stable driver biomarkers on tumor cells which bind highly specific therapeutic molecules. Generally, such biomarkers are normal cell proteins whose genes have acquired specific mutations, giving the cells some form of growth advantage. Thus, the choice of specific therapy must now rely on information obtained by DNA sequencing of these genes, giving rise to the concept of "companion diagnostics". Massive parallel sequencing, more commonly known as "next-generation sequencing" (NGS) enables very precise and sensitive methods for gene mutation analysis, and has now become the gold-standard in molecular diagnostics laboratories. The list of specific genes and their mutations that become targets of novel therapies is rapidly expanding, not only in neoplasia, but also in other areas of medical therapy.

Chronic lymphocytic leukemia (CLL) is the most common human leukemia, accounting for over 40% of all human hematologic neoplasia. A major breakthrough in CLL therapy was the correlation between the mutation status of the variable region of the immunoglobulin heavy-chain gene (*IGHV*), prognosis and therapy (ibrutinib or otherwise) [2]. Molecular diagnosis in this disease expands the notion of "gene mutations causing cancer", since prognosis in CLL relies on the sequence of IGHV without any specific "malignant mutations". Furthermore, specific "stereotyped subsets", determined by a unique IGHV sequences, provide additional information that can change prognosis [3]. Although the mutation status of *TP53* gene has now also become a major determinant of disease progression [4], *IGHV* sequencing remains the most important prognostic indicator.

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ORGANIZATION OF 5S RIBOSOMAL DNA IN THE GENUS *AESCULUS* (FAMILY SAPINDACEAE)

Molecular markers represent a convenient tool for assessing the genetic variability. Due to occurrence in the genomes of all eukaryotic organisms, evolutionary variable intergenic spacer (IGS) of 5S rDNA has been successfully used for the investigation of the evolution of repeated sequences as well as for molecular phylogeny. However, this genomic region still remains not investigated in many groups of plants, e. g. in the genus *Aesculus* (family Sapindaceae), which includes about 13 species distributed in North America, South Asia and the Mediterranean.

In plants, the 5S rDNA IGS can be used to evaluate relationships between closely related species, populations, and sometimes even between individuals. Accordingly, we cloned and sequenced this region for the *Aesculus octandra, Ae. pavia* and *Ae. chinensis* genomes and estimated the genetic distances between these species and other representatives of the family Sapindaceae.

Our data show that the IGS of *Aesculus* species contain sequence motives, which are similar to those ones involved in the 5S rDNA transcription in the species from other families of angiosperms [1]. These motives include putative “TATA”-box, GC and C elements, which are involved in the transcription initiation, as well as an oligo-dT region required for termination.

Besides, the data show that in *Ae. octandra* there is a nucleotide deletion at the 3`-end of IGS that affects the regulatory elements. At the same time, in some clones of *Ae. octandra* there is a sequence duplication in the same position of IGS, which may compensate the deletion.

Between different species of the genus *Aesculus*, the level of IGS similarity ranges from 68.6 to 88.2%. The level of similarity between IGS of genera *Aesculus* and *Acer* ranges from 54.1 to 55.5%.

Hence, polymorphism of the 5S rDNA IGS can be used to study the evolution and taxonomy of the genus *Aesculus.*

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ГЕНЕТИЧНА ХАРАКТЕРИСТИКА АНТАРКТИЧНИХ АКТИНОБАКТЕРІЙ

Скринінг нових біоактивних сполук, головно антибіотиків, не втрачає своєї актуальності. Актинобактерії – продуценти майже 80% відомих на сьогодні антибіотиків [1]. Мешканці екстремальних біотопів, таких як Антарктика, можуть бути джерелом нових сполук. Аналіз геномів антарктичних мікроорганізмів може пролити світло на їхній біотехнологічний потенціал як продуцентів біоактивних речовин, в т. ч. нових.

Метою роботи була генетична характеристика актинобактерій ризосфери *Deschampsia antarctica* (о. Галіндез, Морська Антарктика). Аналіз гену 16S рРНК 43 ізолятів дозволив ідентифікувати представників 5 родів: *Streptomyces, Micromonospora, Kribbella, Micrococcus* та *Umezawaea*. Рідкісний рід *Umezawaea* в антарктичних біотопах ізольовано вперше. Детальний аналіз 11 ізолятів цього роду показав, що ізолят Da 62–02 не утворював клади з жодним описаним видом роду *Umezawaea*. Філогенетична віддаль ізоляту Da 62–02 може вказувати на його приналежність до нового виду.

Геном штаму Da 62–02 складає 11 469 175 п.н. із вмістом Г+Ц 70,62%. Анотація геному дозволила ідентифікувати 10140 генів, 61 ген тРНК, 3 гени рРНК, 22 регуляторні гени та 9 генів некодуючих РНК. Також виявлено гени стійкості до важких металів, гени деструкції ароматичних сполук та гени синтезу вітамінів. За допомогою програми antiSMASH v.6.0 виявлено 49 ймовірних кластерів генів біосинтезу вторинних метаболітів. Серед них по 6 кластерів синтезу нерибосомних пептидсинтаз та лантепептидів, 3 кластери полікетидсинтаз I типу, 8 кластерів синтезу терпенів, 2 кластери синтезу ектоїну та по 1 кластеру синтезу індолів, сидерофорів та олігосахаридів. Зокрема, було виявлено сім кластерів з високою гомологією до вже відомих кластерів генів біосинтезу: бафіломіцин B1 (61%), індигоїдин (80%), еритрепептин (75%), ізоренієретен (80%), геосмін (100%), алкілрезорцінол (100%) та макротерміцин (96%).

Отримані результати демонструють хороший потенціал *Umezawaea sp.* Da 62–02 як продуцента біологічно активних сполук. Подальша характеристика і аналіз послідовності чернетки геному цього штаму дадуть змогу відібрати кластери-кандидати для гетерологічної експресії в штамах-господарях для визначення імовірно нових біологічно активних сполук.

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MUTATION SPECTRA IN *STREPTOMYCES* AS INFERRED FROM GENOMIC ANALYSIS OF NINE *STREPTOMYCES ALBUS* J1074 STRAINS

The genus *Streptomyces* unites Gram-positive bacteria known for their complex differentiation cycle, extensive secondary metabolism, and large linear genomes with high GC content. The latter is a defining trait of the entire class *Actinobacteria*, which *Streptomyces* belong to. There is lively – and unresolved – debate on the origins and consequences of the GC composition in actinobacteria, and in bacteria in general [1, 2]. A better understanding of this issue should improve our knowledge of genome evolution in bacteria and will have a number of biotechnological ramifications. The current intensive investigations and development of genomic sequence databases has put us into position where various conjectures about GC content in streptomycetes can be systematically addressed through analysis of sequencing data gathered within and across species. We used *Streptomyces albus* J1074 as an experimental model to reveal genome-wide spectrum of mutation, which appears to be biased towards elevated GC content. The genomic GC content in streptomycetes varies from 75% to 66.5%, with median value being 72%. The GC content of secondary metabolic genes of *S. coelicolor* is less variable than that of primary metabolic genes, indicative of different selection pressure on these gene groups. Along with selective constraints, the peculiarities of *Streptomyces* DNA mismatch repair might contribute to the skewed GC content of their genomes.

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UTILISATION OF GENOME EDITING METHODS IN DIAGNOSTICS AND GENETIC ENGINEERING, A REVIEW

Recently we have studied the structural organisation of the flax tubulin, actin and cellulose synthase families of paralogous genes and their interactions during the deposition of the cellulose fibres. To further investigate the role of particular isotypes and the possibilities of the genome editing for the flax cellulose quality and quantity we have assessed a number of the novel genome editing methods. We have pointed out 1) the expression activation / repression of the genes of certain isotypes, 2) the sequence of the C-terminal tail, which is responsible for the protein-protein interactions and 3) post-translational modifications, as the three sensitive components of the cellulose synthesis.

Firstly, we suggest that the multiplex ability of a CRISPR-Cas9 system to edit highly homologous target sequences makes it possible to overcome the challenges of target identification in families of paralogous genes with high sequence homology and different or overlapping functions of each isotype, usual for the plant genomes polyploidy. Through the Cas9 system abilities of simultaneous targeted mutagenesis, synergistic or tuneable transcription activation / repression, simultaneous activation / repression of multiple genes it makes possible the modification of either all the paralogous genes at a time or a certain isotype. Deactivated Cas9 (dCas9) can be fused either to a transcriptional activator such as VP64 for overexpression or a transcriptional repressor such as SRDX for downregulation.

Another class of challenges are the regulation of the expression of different isotypes by non-coding RNAs originating from introns and the need to target tissue specific promoters as the expression of certain isotypes is tissue specific. We suggest Cas13– an entirely new Class 2 effector, that acts exclusively on single-stranded RNA and opens a tremendous variety of application in the different fields of regulation of gene expression, RNA knockout, RNA binding and visualization, and RNA base editing and modification. Cas13 is also effective on non-coding RNAs, the ones from the introns, and it also can knock down tissue specific promoters.

Finally, the post-translational modifications, off-target effects, the presence of a protospacer adjacent motif (PAM) can be addressed with the selection of a more convenient genome-editing strategy. CRISPR-Cas systems that rely on synthetic single-guide RNAs (sgRNAs) are generally preferred. Cas12 is a useful alternative to Cas9, based on the recognition of a different PAM motif and the generation of staggered double-strand breaks and may provide a better experimental strategy.

The effectivity of the genetic modifications optionally can be validated by a number of CRISPR-based genetic diagnostic methods. These methods, are based on the Cas9, dCas9, Cas12a and Cas13 are highly accurate.

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СТРУКТУРНА ОРГАНІЗАЦІЯ ТА ПОЛІМОРФІЗМ МІЖГЕННОГО СПЕЙСЕРА 5S тсДНК КАРПАТСЬКОЇ БДЖОЛИ

Природний ареал медоносної бджоли (*Apis mellifera* L.) охоплює Європу, Західну Азію та Африку. Пристосування до локальних умов існування призвели до формування чисельних підвидів та екотипів медоносної бджоли, на основі яких були створені породи та селекційні раси, які використовуються в різних країнах світу. Неконтрольована інтродукція порід медоносної бджоли, які належать до різних підвидів *A. mellifera*, на нові, не характерні для них території є причиною їх гібридизації із місцевими породами/расами, що в свою чергу спричиняє втрату цих аборигенних форм. Тому актуальним є питання збереження генофонду аборигенних форм *A. mellifera,* зокрема тих підвидів, які мешкають на території західної України. З'ясування походження та філогенії місцевих порід медоносної бджоли, їх приналежності до певних підвидів вимагає застосування сучасних молекулярно-генетичних методів. Зокрема, в якості маркерів можуть бути використані ділянки геному, які кодують 5S рРНК (5S рДНК), а саме – міжгенний спейсер (МГС), що входить до її складу.

Матеріалом для дослідження були робочі бджоли двох селекційних рас Карпатської породи, Рахівська та Говерла. Повторювану ділянку 5S рДНК було ампліфіковано за допомогою ПЛР. Надалі ПЛР-продукти клонували у плазмідний вектор, після чого сиквенували. Аналіз отриманих послідовностей показав, що всі досліджувані клони містять МГС, які з обох боків фланковані фрагментами кодувальної ділянки включно з використаними для ПЛР праймерами. Для порівняльного аналізу також використовувались ділянки 5S рДНК, знайдені в базі даних Genbank.

Встановлено, що в геномі *A. m. carnica* присутні щонайменше два класи 5S рДНК, кожен з яких містить декілька структурних варіантів. Геноми двох досліджених рас Карпатської породи та наявний у Genbank зразок *A. m. carnica* відрізняються за наборами таких варіантів, тоді як повторювані ділянки 5S рДНК *A. cerana* є ідентичними в межах геному. Отримані результати свідчать про високий внутрішньо- та міжгеномний поліморфізм 5S рДНК у *A. m. carnica*.

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POLYMORPHISM ANALYZED BY *ME15 / ME16* AND MICROSATELLITE MARKERS IN POPULATION OF *MYTILUS GALLOPROVINCIALIS* FROM THE NORTH-WESTERN REGION OF THE BLACK SEA

An important role in the ecology of the Black Sea is played by filter clams – mussels. Mussels are now a relevant object of fishing and aquaculture. The Black Sea mussels has been classified as *Mytilus galloprovincialis* (Lamarck, 1819) only for morphological features, including using multidimensional morphometry. In investigation of the Black Sea mussel population, it is very important to establish the genetic identity of the species and study the genetic structure of individual groups and populations, and especially their parameters such as the level of heterozygosity and polymorphism. Recently, molecular markers have been used to address controversial issues of taxonomy and to establish the genetic structure of populations.

Our aim was to analyze the genetic polymorphism of the studied groups of mussels of the North-Western region of the Black Sea using microsatellite markers and the molecular marker *Me 15–16* specific for the unique region of the mussel adhesive protein gene.

Samples of mussels were collected from six locations in the North-Western region of the Black Sea: in the Gulf of Odesa (location A coordinates – N: 46°26’28” / E: 30°46’20”; location E – N: 46°22’35” / E: 30°45’7”), in the area of 411 battery (location B – N: 46°22’2” / E: 30°43’45”), near Snake Island (location C – N: 45°15’18’’ / E: 30°12’15’’), in the Sukhyi estuary (location D – N: 46°20’22’’ / E: 30°39’38’’) and the Tiligul estuary (location F – N: 46°40’46’’ / E: 30°9’26’’).

In our work 171 mussels from A (n=37), B (n=25), C (n=26), D (n=24), E (n=29), F (n=30) locations were analyzed by using *Me 15–16* and only the 126 bp allele was detected, therefore we revealed exclusively individuals of *M. galloprovincialis*.

Homozygous genotypes of mussels are found with high frequency in the locations (A, B, C) according to microsatellite analysis and are characterized by alleles of 234 bp, 238 bp, 242 bp at locus *Mch 5*; 202 bp, 210 bp, 214 bp, 216 bp – locus *Mch 8*; 179 bp, 185 bp – locus *MT 203*; 336 bp, 350 bp – locus *MT 282*.

The highest level of observed heterozygosity was determined by the MS locus *MT 282* (55%) in location C, where 7 alleles were detected (PIC=0.76); the lowest – at the MS locus *Mch 5* (7%) in location B, in which 5 alleles were detected (PIC=0.70); absence – at the MS locus *Mch 8* in location C, in which 5 alleles were detected (PIC=0.59).

We do not observe significant differences according to preliminary data of microsatellite analysis and molecular marker *Me 15–16* between mussels caught in different locations.

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ASSOCIATION BETWEEN ALLELIC VARIANTS OF *GLI-B1* LOCUS AND POLYMORPHISM OF MICROSATELLITE LOCUS *TAGLGAP* IN BREAD WHEAT VARIETIES

Gliadins are one of two main wheat storage proteins that form gluten complex and thus determine flour breadmaking properties. Therefore, gliadin genes are an important trait/marker for breeding. Gliadin genes are localized at six main loci on the 1-st and 6-th chromosomes of each subgenome and eight minor. Two classifications of gliadins are developed on the basis of acid PAGE method: by Sozinov and Popepelya [1996] (often used in Ukraine) and by Metakovsky et al. [1995] (international). Every allelic variant in this case is a complex of linked polypeptide genes inherited as one simple Mendelian trait. Due to the complexity of APAGE method, it is possible to detect some allelic variants by PCR method during selection and breeding process. The aim of the study was to explore the polymorphism of microsatellite locus *Taglgap* and to analyze its association with polymorphism of allelic variants of gliadins.

A set of 71 modern Ukrainian bread wheat cultivars and 43 worldwide varieties were analyzed by acid PAGE method according to Poperelya [1989]. A total of 19 allelic variants were detected in worldwide collection and nine in modern Ukrainian collection. The most common for Ukraine were Gli-B1b (p=0,52) and Gli- B1l (p=0,31).

Ten alleles of microsatellite locus *Taglgap* were found using PCR with primers developed by Devos el al. [1995]. Seven alleles – 213 bp, 237 bp, 246 bp, 250 bp, 265 bp, 270 and null were detected for Ukrainian cultivars, and nine alleles were found for worldwide collection – 210 bp, 213 bp, 237 bp, 246 bp, 248 bp, 250 bp, 270 bp, 285 bp and null. 265 bp allele of *Taglgap* locus was unique for Ukrainian wheat collection, and three alleles – 210 bp, 248 bp and 285 bp occurred only in worldwide collection.

We revealed an association between allelic variants of gliadins and alleles of *Taglgap* locus: Gli-B1a allelic variant corresponds to 285 bp allele of *Taglgap* locus; Gli-B1b (Ukrainian and Russian cultivars) and Gli-B1n, Gli-B1q, Gli-B1s – 213 bp; Gli-B1b (Canada, Australia) – 210 bp; Gli-B1c, Gli-B1e, Gli-B1g, Gli-B1f – 237 bp; Gli-B1i, Gli-B1j, Gli-B1k, Gli-B1m, Gli-B1p, Gli-B1o, Gli-B1r – 246 bp; Gli-B1h – 248 bp, 250 bp; Gli-B1d – 265 bp, 270 bp; Gli-B1l – null allele.

Therefore, microsatellite locus *Taglgap* is highly polymorphic and allows distinguishing Gli-B1a, Gli-B1d, Gli-B1h, Gli-B1l allelic variants of gliadins.

Nonetheless, for a number of allelic variants we should analyze polymorphism of more loci and more molecular markers to detect them by PCR.

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KASP-АНАЛІЗ ІНТРОГРЕСИВНИХ ЛІНІЙ М'ЯКОЇ ПШЕНИЦІ ЗА ДОПОМОГОЮ МАРКЕРІВ СПЕЦИФІЧНИХ ДО 1R ХРОМОСОМИ ЖИТА

1RS хромосома жита (*Secale sereale* L.) широко застосовується при інтрогресивній гібридизації з м'якою пшеницею (*Triticum aestivum* L.) для перенесення генів стійкості до борошнистої роси (*Pm8*), стеблової (*St31*), листової (*Lr26*) та смугастої (*Yr9*) іржі (Singh et al., 2015), а також для підвищення врожайності та толерантності пшениці до несприятливих кліматичних умов (Howell et al., 2014). Наявність в 1RS хромосомі *Sec-1* локусу, що кодує запасні білки жита секаліни та втрата гліадинових і глютенінових локусів з заміщених 1AS або 1BS хромосом пшениці негативно впливає на хлібопекарські характеристики борошна (Dhaliwal, MacRitchie, 1990).

Джерелом 1RS хромосоми для більшості сортів та ліній м'якої пшениці, що має 1RS.1BL транслокацію є сорт жита Petkus (2x) (Rabinovich, 1998). Іншим шляхом створення 1RS.1BL транслокації є гібридизація з використанням тритикале (8x) (Tsunewaki, 1964). Аргентинський сорт жита Insave F.A. та по-

хідний від нього сорт пшениці Amigo отриманий в 1976 році став основним донором транслокації 1RS.1AL (Sebesta et al., 1978).

Метою нашого дослідження є ідентифікація молекулярно-генетичного поліморфізму 1RS хромосоми з різних джерел за допомогою молекулярних SNP маркерів в спеціально створених інтрогресивних лініях і сортах пшениці.

Після молекулярного-гентичного аналізу з залученням 8 маркерів специфічних до 1RS і 15 маркерів специфічних до 1BS та електрофорезу запасних білків м'якої пшениці (гліадинів та глютенинів) з 63 оригінальних пшеничних ліній BC_1F_8, що були отримані І.І. Моцним від схрещування та беккросування інтрогресивної лінії Erythrospermum 125/03 (E125/03) та мутанта Chinese Spring *ph1b* (CS*ph1b*) було відібрано 11 інтрогресивних ліній, що потенційно мають цікаві хромосомні перебудови.

Для ідентифікації молекулярно-генетичного поліморфізму 1RS хромосоми використовували KASP-аналіз із залученням 15 специфічних SNP праймерів розроблених KWS (Німеччина). Метод генотипування KASP заснований на конкурентній алель-специфічній ПЛР і дозволяє визначити однонуклеотидний поліморфізм в обох алелях, а також інсерцію або делецію певної області.

Результати KASP-аналізу підтверджують наявність/відсутність 1RS.1BL транслокацій та рекомбінацій між 1RS і 1BS у досліджених лініях. За допомогою KASP-аналізу було виявлено поліморфізм у досліджених сортах і лініях. Виявлено 3 групи ліній, що розрізнялися за типам короткого плеча 1RS хромосоми. Також, детектували гетерогенність у деяких зразків інтрогресивних ліній.

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PCR-DIAGNOSTICS OF THE PHOTOPERIOD SENSITIVE *E* GENES IN UKRAINIAN SOYBEAN VARIETIES AND PERSPECTIVE LINES

Short-day plant soybean (*Glycine max* (L.) Merr.) required absence or low sensitivity to photoperiod for adaptation to high latitudes. There are multiple loci known as *E* series or eleven major genes *E1 – E11* and *J* gene, which generally controlled time to flowering and maturity, two traits that are highly influenced by photoperiod [1, 2]. Among these loci dysfunctional alleles of maturity genes *E3* and *E4*, which are phytochrome A genes, are involved in soybean photoperiodic insensitivity. For most genotypes of soybean varieties and lines created in different breeding centers of Ukraine, identification of alleles by allele-specific markers for loci *E3*, *E4* was not performed. Due to the contradictions and ambiguity of the results of our previous analysis with microsatellite markers associated with loci *E3*, *E4* [3], we decided to focus on the analysis of alleles of these genes, using allele-specific markers.

As material were used: cultivars Kobza, Mavka, Geba, Poltava, Romashka, Halyna, Zolotysta, Krynytsia, Femida, Podilska 416, Podiaka, Oksana; control varieties – isoline Harosoy OT 89-5, Vilana, Maple Arrow, Cormoran AC and Ros; 19 lines (F_{8-10}) derivatives from crossing: Oksana x Labrador (5 lines), Mapple Belle x Sreska72 (7 lines), Linia103 x Korada (7 lines); 10 lines obtained by chemical mutagenesis: Oksana M2, Oksana M12, Oksana M13, Zolotysta M16, Zolotysta M20, Femida M29, Femida M32, Podilska 416 M33, Podilska 416 M38, Podilska 416 M40.

Genotyping was performed by using allele-specific DNA markers for *E3* gene: *E3-Mi/ E3-Ha/ e3-tr* alleles and *E4*gene: *E4/e4-SORE-1* alleles, as recommended by Xu et al. [4] and Kurasch et al. [5]. Field experiments were conducted for 3 years on the territory of Vinnitsia region of the Right Bank Forest-Steppe under field conditions of IFAP, 49°13`N (Vinnitsia, Ukraine).

Most varieties were carriers of the dominant alleles *E3* and *E4*. Recessive alleles of *e3-tr* were found only in varieties Zolotysta and Mavka. It should be noted that for variety Heba amplification fragments with primers to *E4* gene or *e4-SORE-1* allele were not detected. Therefore, it seems that this variety is a carrier of other recessive alleles at the *E4* gene.

We found that chemical mutagenesis induced variability in certain loci of the genome. These changes more affected *E3* locus. The dominant allele *E3-Ha* in the original variety Oksana was changed in the mutant lines (Oksana M12, Oksana M13) to recessive *e3-tr* and the similar situation was observed for Podilska 416 variety and

mutant line Podilska 416 M33. We observed restoration of the recessive allele *e3-tr* to the allele of dominant type *E3-Ha* for variety Zolotysta and its derivative lines Zolotysta M16 and Zolotysta M20. We did not observe any changes in the mutant lines at *E4* locus.

Derivatives of Oksana variety had a shortened stage of maturation, and derivatives of Femida variety maturated later than the original variety. The derivative forms of the Oksana variety (lines M2, M12, M13) had significantly higher yields, but no significant difference was observed in the derived lines from variety Femida.

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FEATURES OF MICROFLORA OF THE MAIN BIOTOPES IN VERY LOW BIRTH WEIGHT PREMATURE INFANTS AT INTRAUTERINE INFECTION

The high infection rate of the adult population with viruses and bacteria determines the significant prevalence of intrauterine infections. Intrauterine infection is more likely to cause premature birth, leading to infant death.

The aim of the work is to study the nature of microbiocenoses of the main biotopes in premature infants at intrauterine infection.

38 premature infants (up to 1500 g at birth) hospitalized at the neonatal intensive care unit were enrolled in this study. Microbiological examination using PCR method was carried out. As a material blood, scrapings from oropharynx and nasopharynx were taken for DNA detection of *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum*, *Herpes simplex virus* type I, type II, *Cytomegalovirus*,

Epstein-Barr virus. Detection of microbial DNA was carried out by Real-Time PCR using test systems manufactured by NPF "DNA-Technology".

Intrauterine infection of the oropharynx and nasopharynx was detected in 32 examined samples (84.21 ± 7.57%), while in 29 (90.63 ± 8.02%) the identified pathogens were present in the mixed infection: DNA of *Ureaplasma urealyticum* + *Herpes simplex virus* was detected in 12 samples (37.50 ± 5.74%), *Ureaplasma urealyticum* + *Cytomegalovirus* – in 9 samples (28.12 ± 5.06%), *Ureaplasma urealyticum* + *Mycoplasma hominis*, as well as *Herpes simplex virus* + *Cytomegalovirus* – in 8 samples (28.12 ± 5.06%, respectively). In the blood, the investigated pathogens were detected in 9 patients (23.68 ± 4.64%): DNA of *Cytomegalovirus* – in 2 (22.22 ± 4.67%), *Herpes simplex virus* + *Cytomegalovirus* – in 4 (44.44 ± 6.53%), *Ureaplasma urealyticum* + *Cytomegalovirus* – in 1 (11.11 ± 3.31%), *Ureaplasma urealyticum* + *Herpes simplex virus* – in 2 children (22.22 ± 4.67%).

The main microbiological factors of intrauterine infection in very low birth weight premature infants and in extremely low birth weight premature infants are *Ureaplasma urealyticum Herpes simplex virus* types I, II, *Cytomegalovirus* and *Mycoplasma hominis,* at which pathological formation of microflora of the main biotopes occurs, the molecular genetic study of which is necessary for further correction of the formation of the normal flora of the main biotopes of the child's organism.

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RESULTS OF BREAST MILK TESTING FOR HERPESVIRAL INFECTIONS BY PCR

Early diagnosis of perinatal infections is one of the problems of Obstetrics and Neonatology. A standard examination of pregnant women is carried out at antenatal clinic and involves antibody detection to *Cytomegalovirus* (CMV), *Herpes simplex virus types* I, II (HSV) in the blood by enzyme immunoassay. The incidence of congenital herpes and cytomegalovirus infections among newborns registered in the neonatal department is 0.6% per year. In retrospective analysis, the incidence rises to 7.4% per year. Registration of these infections most often occurs in the department of children's infectious diseases.

The aim of the work is to study the possibility of using the PCR for examining the breast milk when making the diagnosis "congenital herpes and cytomegalovirus infection" in infants.

We examined 21 infants (first year of a baby's life) at the age of 1–4 months receiving life-saving therapies, as well as their mothers (n = 21). As a material, breast milk was taken from the mothers, venous blood was taken from infants, scraping of

epithelial cells from the oral cavity was taken from infants. In the studied biological material, DNA detection of *HSV*, *CMV* and *Epstein-Barr virus* (*EBV)* was carried out by Real-Time PCR.

Breast milk infection was detected in 19 mothers (90.48 ± 8.56%): mono-CMV infection was detected in 6 (31.57 ± 5.45%), *HSV* – in 2 samples (10.53 ± 3.21%); mixed infection *CMV* + *HSV* – in 9 (47.37 ± 6.56%), *CMV* + *EBV* – in 2 samples (10.53 ± 3.21%). In oropharyngeal scrapings, infection was detected in 17 infants (80.95 ± 8.20%): *CMV* DNA was detected in 5 (29.41 ± 5.28%), *HSV* – in 2 samples (11.76 ± 3.39%); CMV + HSV – in 9 (52.95 ± 6.94%), CMV + EBV – in 1 (5.88 ± 2.41%) sample. In the study of blood, infection was confirmed for 14 samples (66.67 ± 7.57%): *CMV* DNA was detected in 4 (28.57 ± 5.24%), *HSV* – in 2 samples (14.29 ± 3.74%), *CMV* + *HSV* – in 7 (50.00 ± 6.82%), *CMV* + *EBV* – in 1 sample (7.14 ± 2.66%).

The high degree of infection of infants in the 1st year of life, as well as their mothers, with the CMV virus makes it possible to consider this pathogen as the main cause of intrauterine infection. The studies prove the need for a parallel examination of infants in the 1st year of life and their mothers to establish the source of infection.

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CLINICAL IN-HOUSE TEST SYSTEM FOR DETECTING VIABLE FORMS OF CHLAMYDIA TRACHOMATIS, CHLAMYDIA PNEUMONIAE AND MYCOPLASMA PNEUMONIAE IN SYNOVIAL FLUID

Etiological diagnosis of joint diseases is based on the use of PCR to detect the DNA of arthritogenic pathogens. In this case, the test of cure is carried out 30–45 days after the last day of taking antibacterial drugs, which is very critical and unacceptable in present-day Orthopedics.

The aim of the work is to assess elimination efficiency of *Chlamydia trachomatis (C.tr.), Chlamydia pneumoniae (C.pn.), Mycoplasma pneumoniae (M.pn.)* using an in-house method for detecting RNA pathogens in the synovial fluid of patients with gonarthrosis.

68 patients with knee arthropathy of inflammatory etiology were examined: group 1 (n = 19) – patients who previously had *C.tr* DNA; group 2 (n = 17) – patients with *C.pn* DNA; group 3 (n = 15) – patients with *M.pn* DNA detected; group 4 (n = 17) – patients, for which the DNA of these pathogens was not detected.

At the first stage, we selected specific pairs of primers and probes, optimized the composition of the amplification mixture and the temperature profiles of the reaction. For groups 1–3, a 100% coincidence was found between the results of the presence of DNA and RNA pathogens; in group 4, *C.tr.*RNA. (n = 2), *C.pn*. (n = 1) and *M.pn*. (n = 1) was detected. 10 days after the end of antibiotic therapy RNA *C.tr., C.pn., M.pn.* was not detected in any of the samples, while DNA continued to be detected in 41 samples, after 1 month – in 18 samples. At the same time, the results for the detection of RNA and DNA were completely identical only 3 months after the end of antibiotic therapy.

The developed NASBA-PCR method for the detection of viable forms *C.tr., C.pn., M.pn.* can be used both in the complex diagnosis of inflammatory gonarthrosis of unspecified etiology, and for monitoring the effectiveness of antibacterial pharmacotherapy 10 days after the last day of drug intake.

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TECHNOLOGY OF OBTAINING PLATELET-RICH AUTOPLASMA FOR INTRA-ARTICULAR ADMINISTRATION

The production and clinical use of autoplasma enriched with platelet growth factors is a promising area of present-day Traumatology using cellular technologies, the use of which will allow for pathogenetically based therapy aimed at stimulating regenerative and reparative processes in the joint cavity.

The aim of the work is to optimize the technique for obtaining platelet-rich autoplasma for intra-articular use in patients with knee arthropathies.

The blood serum of 15 patients with gonarthrosis was used as a biological material. The control group consisted of 15 apparently healthy individuals matched by sex and age, at the same time, the platelet count in the study groups did not differ statistically.

12 different modes of centrifugation of peripheral blood samples were tested: the number of revolutions (g) and time (min). For the first centrifugation, the 400 and 500 g modes were tested for 2–5 minutes, for the second – 1500, 2000 and 2500 g for 2–5 minutes. The upper layer of plasma was removed, and the lower layer was used to assess the number of platelets. It was found that there were no statistically significant ($p > 0.05$) differences in the content of the number of platelets when used in primary centrifugation at 400 g and 500 g and a time of 2–5 minutes. Centrifugation at 2500 g, regardless of the exposure time, also did not have a significant ($p > 0.05$) effect on the platelet count. Centrifugation at 1500 g produces autoplasma with a higher platelet count compared to centrifugation at 2000 g.

The optimal mode of obtaining platelet-rich autoplasma has been established; centrifugation 500 g 4 min + 1500 g 3 min, the final platelet count is 1859 (1647/2038) x 10^9/ L. The use of a drug with a platelet content of more than 2000 x 10^9/ L is not recommended for use due to the possibility of causing an inhibitory effect on the process of tissue regeneration or cell proliferation.

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MICROBIAL INFECTION OF THE COLON AS A FACTOR IN THE DEVELOPMENT OF DIVERTICULAR DISEASE, ITS COMPLICATIONS AND RELAPSES

The most common cause of the formation of diverticular disease (DB) is a microbial factor; at the same time, in 7–42% of patients there is a recurrent nature of the course of the disease, the risk of relapse is 3–4% during the first year and increases to 90% in the first 5 years from the moment of the primary episode.

The aim of the work is to identify microbiological risk factors for the development of DB, its complications and relapses on the basis of an assessment of the translocation of the colon microflora into the mucous membrane and lymph nodes of the mesocolon.

99 patients with DB of the colon were examined: group 1 (n = 33) – patients with uncomplicated DB; group 2 (n = 35) – patients with complicated DB; group 3 (n = 31) – patients with recurrent DB.

Biopsy specimens of the diverticula and lymph nodes of the mesocolon were used as biological material. PCR method was used to identify the DNA of *Enterobacteriacea (Ent.), Staphylococcus species (St.spp.), Streptococcus species (Str.spp.); Staphylococcus aureus (St.aur.), Herpes simplex virus types I, II (HSV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV)*.

Bacterial lesions of the colon were found in 93.94 ± 8.05% (n = 31) cases of patients in group 1, 96.77 ± 8.23% (n = 30) of cases in patients in group 3 and 100% of cases in patients in group 2; as well as viral infection of both the tissues of the colon (in 45.71 ± 6.20% (n = 16) cases of patients in group 2) and lymph nodes of the mesocolon (in 11.43 ± 5.29% (n = 4) of cases of patients group 2 and in 80.00 ± 7.80% (n = 24) cases of patients in group 3). It was found that when detecting DNA of *St. spp.* DNA detection frequency *St.aur.* was 33.33 ± 5.60% (n = 6) for group 1, 44.00 ± 6.26% (n = 11) for group 2 and 31.25 ± 5.45% (n = 5) for group 3.

In uncomplicated DB, tissue infection of diverticula with opportunistic microflora is observed. In complicated DB, there is a combined bacterial-viral infection of the tissues of diverticula with a viral lesion of the lymph nodes of the mesocolon. Recurrent DB is characterized by the presence of bacterial tissues infection of diverticula and viral infection of the lymph nodes.

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OPTIMIZATION OF *KLEBSIELLA PNEUMONIAE* MOLECULAR-GENETIC IDENTIFICATION METHOD IN BIOLOGICAL MATERIAL OF PATIENTS WITH SEPSIS AND IN BACTERIAL CULTURES

One of biggest challenges modern healthcare continue to face is mortality related to severe pneumonia and sepsis in patients. Annually sepsis affects 18–31.5 million people worldwide and takes 6–8 million lives. Gram-negative bacteria play the key role in the etiology of nosocomial sepsis. *Enterobacteriaceae* is a dominant family of Gram-negative bacteria. Of the pathogenic *Klebsiella* species, *K. pneumoniae* is the most clinically important [1, 2].

To achieve rapid study results when establishing the etiology of sepsis, it is urgent to develop rapid methods for identifying bacteria based on the use of a molecular genetic method.

The aim of the work is to optimize molecular genetic identification method of *K. pneumoniae* for detecting the DNA of the pathogen in biological material and bacterial cultures.

At the first stage of the study, the optimal set of reagents for the isolation of microbial DNA was selected based on the determination of the concentration and purity of the isolated DNA (NanoDrop 1000, Thermo scientific, USA). Significantly higher values, comparable to those of the reference method (phenol-chloroform extraction method), were established using the ArtDNA light reagent kit (ArtBioTech, the Republic of Belarus). Sequences of primers and probe were then selected to detect DNA *K. pneumoniae*. At the next stage, the composition of the amplification mixture and amplification programs were optimized. In the course of optimizing the composition of the amplification mixture, various addition concentration of primers, DNA and Mg2+ ions were tested.

An improved method for molecular genetic identification of *K. pneumoniae*, based on the use of specific primer pairs, optimized composition of the amplification mixture and amplification programs, allows the identification of the pathogen DNA in the biological material of patients with sepsis and bacterial cultures.

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MOLECULAR-GENETIC IDENTIFICATION OF POLYMORPHIC VARIANTS OF *CYP1A1, CYP2E1, CYP2D6* GENES IN DIFFERENT BIOLOGICAL MATERIAL

Research and study of factors that can influence the pharmacokinetics or pharmacodynamics of drugs are the topical directions in modern medicine [1].

The cytochrome P450 (CYP) system is a family of enzymes that are responsible for the oxidative metabolism of endogenous and exogenous compounds. Individual differences in the allelic profile of genes that control the synthesis of enzymes of biotransformation of xenobiotics cause differences in the levels of expression and activity of the enzymes controlled by them, the phenotypic manifestation of this is the variability of the individual sensitivity of a particular organism to the effects of xenobiotics [2].

The aim of the work is to study the possibility of using biological material obtained by a non-invasive method (scrapings of epithelial cells from the oral cavity) to identify polymorphic variants of the CYP1A1, CYP2E1 and CYP2D6 genes.

Peripheral blood taken from 31 healthy volunteers and scrapings of epithelial cells from the oral mucosa were used as biological material. After DNA isolation, the composition of the amplification mixture and amplification programs were optimized for gene fragments *CYP1A1, CYP2E1, CYP2D6*, then restriction analysis was performed using the enzymes StyI, Hinc II, BseMI, MspI, Pst I, DraI, RsaI, BstNI (Thermo Scientific). The coincidence of the results of detecting polymorphic variants of the *CYP1A1, CYP2E1, CYP2D6* genes in various biological material of patients (blood and scraping of epithelial cells) was 100%.

Molecular genetic identification method makes it possible to reliably identify polymorphic variants of the CYP1A1, CYP2E1, CYP2D6 genes both in the blood and in scrapings of epithelial cells from the oral cavity.

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DESIGNING AN EXPERIMENTAL SAMPLE OF AN EIA-TEST SYSTEM FOR DETECTING ANTIBODIES TO THE HEPATITIS E VIRUS IN HUMANS

In the Republic of Belarus, there is a tendency for the growth of the circulation of hepatitis E virus (HEV) in the population. The development of domestic EIA test systems will contribute to the expansion of the share within the domestic market by creating a competitive national product. The aim of the study is to develop an experimental sample of a test system capable of detecting antibodies to HEV in human blood serum by means of the EIA method.

To test the developed test system, the blood serum of 30 patients diagnosed with HEV was used as a biological material, and 30 healthy donors, diagnosed negative for HEV RNA without IgG antibodies, were used as a negative control.

For the production of the test system, genetically engineered polypeptides with the antigenic sequence VGE1 conjugated with beta-galactosidase were used. The optimal concentration of polypeptides for sorption of ORF-2 and ORF-3 on polystyrene plates is 8 mcg/ml in a 0.005 M carbonate buffer pH 9.5. In the group of patients diagnosed with HEV using the developed test system, IgG antibodies to HEV were detected in 28 (93.33±8.20%) patients. No IgG antibodies to HEV were detected in the control group. Diagnostic sensitivity and specificity were 93.33% and 100%, the prognostic value of positive and negative test results was 93.33% and 100%, respectively; diagnostic efficiency was 96.67%.

The developed experimental sample of a diagnostic test system capable of detecting IgG antibodies to HEV in blood serum by means of the EIA method is a worthy analogue of diagnostic test systems produced abroad, on par with them in technical, analytical and diagnostic characteristics, and can be recommended for subsequent production and use in the market of diagnostic services of the Republic of Belarus by health service.

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CREATION OF A LABORATORY SAMPLE OF AN EIA-TEST SYSTEM FOR DETECTING ANTIBODIES TO THE HEPATITIS E VIRUS IN ANIMALS

Viral hepatitis E (HEV) is a zooanthroponotic infection, however, to date, the full range of mammalian carriers has not been fully determined, and screening studies have not been conducted to establish the infection of animals, which may be due to the lack of diagnostic test systems on the domestic market. The aim of the study is to develop an experimental sample of a test system capable of detecting antibodies to HEV by means of the EIA method in the blood serum of pigs.

As a biological material, the blood serum of pigs diagnosed with HEV (n=30) positive for HEV RNA were used, as a negative control (OK) – the blood serum of 30 healthy pigs in which HEV RNA and IgG antibodies to HEV were not detected.

It was found that an increase in the conjugate concentration to 0.3 micrograms/ml led to a significant increase in the optical density of OK, that is, to a decrease in the diagnostic sensitivity (DF) (83.33%) of the test system, in which slightly positive samples (n=5, (16.67±3.98%)) were detected as negative. A decrease in the conjugate concentration to 0.01 mcg / ml led to a decrease in DH to 73.33%. When using a conjugate concentration of 0.1 micrograms/ml, almost complete alignment of the results obtained using the developed test system was established compared to the commercial variant (DH 90.00%). In this regard, high DC is the main criterion for screening studies.

The developed experimental sample of a diagnostic test system using a conjugate at a concentration of 0.1 micrograms / ml, which has high DH (90.00%) and specificity (100%), can be used in screening studies to identify seropositivity of pig livestock, including the process of pooling samples of biological material, without the loss of effectiveness.